bilayer as two abutted monolayers, each with a neutral surface. The constraint imposed by mathematically placing two monolayers in apposition causes minimal energy to be larger than that predicated by (incorrectly) assuming that the elastic properties of a bilayer can be quantitatively captured through a single surface. Independent of pore size, the deformation of tilt did not appreciably affect elastic energies; in other words, membrane splay dominates elastic energies. For small radii, shapes of minimal energy were close to the shape of a catenoid. For large pores, however, deviations of minimal energy shapes from catenoids were large, resulting from the necessity that the membranes be parallel and the separation between them fixed at distances far from the rim of the pore. Energies for minimal shapes were 15-60kT less than the energy of the toroidal shape for pore radii in the range of 2-16 nm and for initially parallel membranes that were separated by 2-4 nm. For the smallest pore possible (i.e., an initial pore), a toroidal geometry overestimated the minimal energy by 30 kT. For pores with radius larger than length, membrane separation near the rim of the pore exceeds the distance between the parallel membranes. These shapes of minimal elastic energy can now be used to calculate fusion pore dynamics.

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Free Energy Landscapes of Vesicle Fusion by Umbrella Sampling MD Simulations

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Despite intensive investigation, the energy landscapes governing membrane fusion in vitro and in vivo remain uncertain. A plethora of factors including small molecules, ions, fusion proteins, and osmotic pressure gradients are known to influence fusion rates, but these perturbations only hint at the underlying molecular mechanisms.

The barriers and metastable structures that characterize fusion free energy landscapes are inherently difficult to resolve atomistically due to the fluid, disordered nature of membranes. These pathways are also difficult to access with molecular resolution simulations, namely molecular dynamics (MD), due to the time scales associated with spontaneous fusion and the lack order parameters capable of driving fusion progress through high energy intermediates.

To address this challenge, we have developed a novel umbrella sampling method paired with an order parameter capable of driving and controlling fusion progress. Our initial results for 20 nm POPC vesicles give a barrier of 43 kBT along a pathway beginning as a metastable stalk, proceeding over a barrier with a hemifused structure and then ending as an opened fusion pore. Though marginally metastable, the hemifusion diaphragm does not expand, likely due to the small vesicle size and the lack of a lipid reservoir, but instead either reverts back to a stalk or proceeds forward to form a fusion pore.

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Local Stresses in Fusing Membranes from Molecular Simulation

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Membrane fusion involves transient and non-uniform stresses on the participating membranes. It is believed that these stresses help drive evolution of lipidic fusion intermediates and determine fusion pathways and outcomes. It has also been shown both via experiments and molecular dynamics simulations that lipid composition can dramatically affect fusion kinetics and efficiency. We have developed a means to measure locally resolved pressure in molecular dynamics simulations and implemented it in the GROMACS software. We use this to measure pressure stresses on highly-curved fusion intermediates. The non-uniform, fluctuating, and spatially curved nature of these intermediates makes measurements challenging; we utilize techniques from computational geometry to assist convergence in our measurements. We interpret our findings in the context of prior fusion theories of lipidic stalk formation, hemifusion interstitial energy, and pore formation. We also examine local membrane pressure changes near fusion peptides.

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A Molecular Mechanism of Lipid Membrane Fusion

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Membrane fusion is an essential molecular event involved in many cellular processes, such as exocytosis, endocytosis, intracellular vesicle trafficking, fertilization, and viral infection to target cells. In spite of extensive studies of membrane fusion, however, the basic molecular mechanisms in biological systems are not well understood. Probably, it is due to the complex nature of biological membranes and the variety of possible molecular pathways for membrane fusion. We have studied the membrane fusion process, particularly ion-induced membrane fusion. Biological membrane fusion seems to occur with either ion-induced or non-ion-induced membrane process, particularly the later case is for virus membrane fusion system. Dr. Chernomordik and his co-workers have studied on non-ion-induced lipid membrane fusion and developed the so-called ''Stalk-intermediate model'' before total membrane fusion. That fusion model has been well received by many membrane fusion investigators, particularly in the virus fusion field. Stalk formation between two lipid membranes may occur due to undulation of lipid molecules or local binding of the lipid bilayers, which results in the formation of a local region of outer monolayer fusion. The stalk hypothesis can be described by macroscopic models treating bilayers and monolayers as homogeneous elastic surfaces. We have also studied non-ion-induced bilayer membrane fusion. Our membrane fusion theory is based on the interaction energies between the two membranes due to alternation of the membrane surface properties, e.g., hydrophilicity and hydrohobicity of interacting membranes, and then lipid membrane close approach and due to membrane curvature. Although the membrane interaction processes are different between the two models, these membrane fusion properties are the same as those of our ion-induced lipid membrane fusion.

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Influence of pH and Side-Chain Negative Charge on the Behavior of Designed Transmembrane Peptides in Lipid Bilayers

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GWALP23 (acetyl-GGALW^{5}LALALALALALALW^{19}LAGA-amide) is a favorable model peptide for investigations of single-residue effects on protein-lipid interactions and the properties of membrane-spanning helices (J. Biol. Chem. 285, 31723). GWALP23 has favorable properties in bilayer membranes because the peptide exhibits only limited dynamic averaging of NMR observables such as the ^{2}H quadrupolar splitting or the ^{15}N-^{1}H dipolar coupling (Biophys. J. 101, 2939). To investigate the potential influence of negatively charged side chains upon system properties, we have substituted a single Leu residue with Glu at different positions and incorporated specific ^{2}H-Ala labels in the core of the single-Trp peptide Y^{5}GWALP23 (see Biochemistry 51, 2044). Solid state ^{2}H NMR experiments were used to examine the peptide orientation and dynamics as functions of the lipid bilayer thickness and pH in hydrated lipid bilayer membranes. We observed well defined ^{2}H quadrupolar splittings for Y^{5}GWALP23-E16 in the pH range from 4.0 to 8.2, suggesting that the peptide helix is well oriented in DOPC lipid bilayers. The glutamic acid residue, though protonated, seemed to confer multi-state behavior at pH 2.5, and the resulting populations exhibited slow exchange on the NMR time scale. The deprotonation of E16 at pH 8.2 did not have any effect on the peptide orientation, perhaps suggesting that the close proximity of E16 to W19 (on the next helical turn) could provide stability to the peptide helix. We are also studying the peptide-lipid behavior when Glu is substituted in position 12 and/or 14, individually or together.

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Functional Consequences of Incomplete Hydrophobic Matching at TM1 of the LeuT Transporter

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The Leucine Transporter (LeuT) is the prototype for structure-function studies of mammalian Neurotransmitter: Sodium Symporters such as DAT, SERT and NET, the transporters for dopamine, serotonin, and norepinephrine, respectively. Its functional sensitivity to the environment, i.e., membranes or detergents in various compositions, has engaged much recent research. As the role of the environment in the function and organization of transmembrane proteins has been shown to involve hydrophobic mismatch, we investigated the membrane deformation and extent of hydrophobic matching for LeuT with the recently described hybrid Continuum-Molecular Dynamics (CTMD) method that combines elastic continuum formulations with an atomistic description of the lipid-protein interface from molecular dynamics simulations. The analysis was performed for functionally relevant conformations of LeuT embedded in two different model membranes: a POPC lipid bilayer and a model bacterial bilayer composed of a 3:1 mixture of POPE and POPG lipids. In both bilayers we found significant membrane thinning and water penetration near the membrane-facing Lys288 of TM7, a positively charged residue embedded